Applicants have addressed this point by amending the claims to recite that the host being treated according to the present methods is one "capable of generating an immune response". It is well-known and established in the art that patients with, for example, late stage tumors, viral infections, or the like, can demonstrate general immunosuppression. The presence or absence of such generalized immunosuppression, and the general immune status of patients, is routinely determined as a part of the medical care of these patients. Clearly, a patient generally incapable of mounting an immune response would not be a candidate for the immunotherapeutic methods taught in the present invention; significantly, such a patient would most likely not be a candidate for *any* form of immunotherapy currently practiced in the art. Applicants' methods are now specifically directed to hosts capable of generating an immune response; the claims have been amended to reflect that only certain hosts would be candidates for the claimed treatment. The immune response elicited as a result of the present methods would therefore be expected to occur in a host having the capability of mounting an immune response. As the general immune status of candidates for immunotherapy, or any other form of anti-cancer or anti-viral therapy, is routinely determined, such a determination relevant to the applicability of the methods of the present invention cannot be considered undue experimentation.

The Office Action also states that the claims are not enabled based upon the allegedly broad recital that the present treatment methods "elicit an immune response". On page 4, the Office Action appears to equate Applicants' claimed methodology of eliciting an immune response with providing protective immunity. As also noted on page 4 of the Office Action, the specification discloses that the present invention stimulates CTL mediated immunity which in turn promotes direct destruction of specific neoplastic cells or virally infected cells within a host. As a point of clarity, therefore, Applicants note that the present invention is directed to immunotherapy that can be either prophylactic or therapeutic; that is, the present methods result in the destruction of tumor cells, other neoplastic cells and virally-infected cells regardless of whether they are subsequently introduced (protective) or already exist in the host (therapeutic). Applicants' claims, as amended, are directed to methods for treating a host capable of generating an immune response by utilizing a novel and non-obvious method

which results in the elicitation of an anti-tumor or anti-viral immune response in the host; this response causes neoplastic cells and virally-infected cells to be destroyed. Applicants respectfully submit that the amendment to the claims reciting destruction of cells addresses the point raised in the Office Action regarding the alleged overbreadth of "eliciting an immune response". The specification supports these amendments.

For example, as is discussed throughout the specification, the present invention is directed to therapeutic or prophylactic genetic immunization of a mammalian host. (See, for example, page 5, lines 2-3, 8-9 and 24-25.) That the present invention is directed to therapeutic or prophylactic genetic immunization and the treatment or prevention of tumors or viral infections is also discussed on page 13, lines 12-13, 17-18, and 24-25. As is further described on page 13, line 24 through page 14, line 13, the present genetic immunization methods can be utilized for either treatment or prevention of tumors or viral infections; presentation of proteins or protein fragments specific to an affected cell provides the substrate for generating an antigenic peptide for presentation to T-lymphocytes by the MHC class I pathway. This presentation through the MHC class I pathway stimulates CTL production and, in turn, promotes destruction of the affected cell. Thus, the destruction of neoplastic cells or virally infected cells is effected by the present method.

The examples also support the claims, as amended. The examples presented by Applicants specifically show the effectiveness of the present methods in protective immunity; Applicants respectfully submit that one skilled in the art would accept this showing as being indicative of both protective immunity (*i.e.*, vaccination) in patients without the disease and therapeutic immunity (*i.e.*, treatment) in patients already afflicted with an illness. Efficacy of treatment is typically demonstrated in the immunological art first through use of a tumor challenge model in which the subject has not been previously exposed to the illness, such as in the examples presented in the present application; this methodology is widely accepted in the art because the mechanism that the host immune system undergoes upon treatment according to the present methods is the same, regardless of whether the host receives a post-inoculation challenge, or is already afflicted with a disease; in other words, the same mechanism which prevents growth of a tumor

or onset of a viral infection in a post-tumor challenge is the same mechanism which causes destruction of tumor cells or virally infected cells in an animal afflicted with the disease prior to the treatment. As evidence of the acceptability of this post-tumor challenge model, Applicants note the results presented by Mayordomo et al., *Nature Medicine*, 12(1): 1297-1302 (1995), a copy of which is enclosed. Mayordomo shows that immunization (with bone marrow derived dendritic cells prepulsed with tumor-associated peptides) can elicit a T lymphocyte response that protects against a subsequent tumor challenge; essentially the same methods were then demonstrated as being effective in causing tumor regression in an animal that already had tumors. In addition, U.S. Patent No. 5,643,578, also enclosed, is directed to methods for immunizing a vertebrate against an infectious agent, or for use in anti-cancer therapy. The only examples offered in support of the claimed methods involved a post-immunization challenge. These references demonstrate the acceptability of Applicants' data by those skilled in the art and by the U.S. Patent and Trademark Office.

In addition, the present examples provide results that would be accepted by those skilled in the art as demonstrating the efficacy of the present methods of eliciting either an antitumor or antiviral immune response. It is well-known in the art that CTLs are critical effector cells capable of recognizing and killing tumor cells or virally infected cells. The methods of the present invention result in the production of antigen-specific CTLs. The specificity of these CTLs depends on the nature of the immunizing antigen, whether that antigen comes from a tumor cell or a virally infected cell; immunization against an antigen expressed by tumor cells would generally result in recognition and lysis of tumors expressing that antigen and immunization against a viral antigen expressed by virally infected cells would generally result in recognition and killing of cells infected by the virus. Thus, the same mechanism is at work regardless of whether the antigens are tumor based or virus based.

For all of the above reasons, Applicants respectfully submit that evidence accepted by those skilled in the art supports the present methods of destroying neoplastic and virally-infected cells in a host capable of generating an immune response.

The Office Action appears to further state that the demonstration using the OVA antigen is not sufficient to support the broad recital of an antigenic protein or antigenic protein fragment. As noted by Applicants in previous responses, the OVA antigen was specifically selected because of its widespread use and acceptance in the immunological art as being representative of all antigens. Applicants have provided numerous other refereed articles in which the OVA antigen was used, and the results obtained therefrom were accepted by skilled artisans. (See Preliminary Amendment.) OVA, as well as other antigens and peptides are known to stimulate immune responses, both in methods such as those of Applicants and also in other immunotherapies. For example, Mayordomo utilized various peptides in their immunological studies, as did the inventors in the '578 patent. The Tüting article, cited in Applicants' Preliminary Amendment but not addressed in the Office Action, provides perhaps the best evidence to support Applicants' position. Tüting *clearly* shows that the present methods are effective in expression of at least five other melanoma antigens and in the subsequent stimulation of CTLs in response to these antigens. The methods utilized in the Tüting article are essentially the same as those reported by Applicants in their examples. (See Tüting, page 1140, column 2 "Particle Mediated Gene Transfer to DC", a copy of which is enclosed.) Moreover, Tüting reports that expression of these antigens by <u>human</u> antigen presenting cells elicits tumor-reactive CTL. (See Tüting, page 1142, section bridging columns 1-2.) Thus, Applicants' claimed methodologies are effective in eliciting CTL responses when using a variety of antigens and when using human cells. There can be no better evidence of the enablement of the present invention than the fact that others have reproduced Applicants' methods and confirmed that the results as claimed are achieved.

The Office Action states that the present claims "even read on protective immunity to Hepatitis C virus." (See Office Action, page 5.) Citation is made to the Nakano et al. article, which allegedly teaches that different routes of injection of the Hepatitis C virus can result in different quantitative and qualitative humoral immune responses in mice and states that "it remains to be seen whether nucleic acid-based immunization could result in the induction of such antibodies in a susceptible animal model." (See Office Action, page 5.) Applicants respectfully submit that Nakano does not defeat the patentability of the present invention. First

of all, there is no indication that the Nakano reference teaches the same methodology taught by Applicants. Second, the fact that Nakano speculated as to whether nucleic acid-based immunizations would work does not mean they don't work. Finally, as is discussed below, Applicants' methods are not dependent on the induction of antibodies, but rather result in the stimulation of a CTL response.

The Office Action cites to the Orkin et al., Hanania et al. and Barry et al. references for such propositions that relevant animal models must be presented, that certain patients become immunocompetent, and that various factors must be taken into account in determining appropriate methodologies to treat various patients.

As noted previously by Applicants, the mouse model, particularly one using the OVA antigen, is well-accepted in the art. Thus, Applicants have proven their methodology using an art accepted mouse model. In addition, Applicants demonstrated, in the Falo Declaration dated May 5, 1998, that human cells are capable of uptake and expression of a marker gene according to the present methods. Significantly, Tüting demonstrated the claimed methods work using human antigen presenting cells and five different tumor antigens; the dendritic cells transfected with these antigens showed specific CTL responses *in vitro*. In addition, the Kim and Weiner article, also cited in the Preliminary Amendment but not addressed in the Office Action, demonstrates the efficacy of DNA gene vaccination using a DNA envelope expression cassette first in small animals, then in primates, and now in human subjects; protective immunity was observed at all levels. (See Kim and Weiner, pages 179-182, a copy of which is enclosed). Applicants respectfully submit that the *in vivo* mouse data coupled with the *in vitro* human data, as well as evidence presented by others in the immunotherapy art, demonstrates the efficacy of the claimed methods, and provides correlatable results that would be accepted by those skilled in the art.

With regard to the Hanania reference, the fact that certain treatments and therapies are ineffective in hosts which are immunocompetent has been addressed by the claim amendment reciting that the hosts treated by the present methods are specifically those capable of generating an immune response.

With regard to the Barry reference, it is well-established that methods of genetic immunization, as well as other immunizations, therapeutic treatments,

and the like, depend on various factors which vary from patient to patient, depending on, for example, the type of illness being treated, the severity of the illness, the response seen by the patient, etc. Accounting for such factors in the treatment of a host is well within those skilled in the art; surely the Applicants cannot be required to recite how every conceivable variation of host would be treated according to the methods of the present invention. Applicants submit that the recital to hosts "capable of eliciting an immune response" further addresses the issues raised in conjunction with the Barry article.

Finally, the Office Action notes that Applicants have failed to provide sufficient guidance and direction for carrying out the claimed methods in "any and all mammalian hosts against any and all types of neoplastic or virally infected cells." (See Office Action, page 8.) As noted above, not every treatment works in every patient; that applies to all treatments, Applicants' or others, for numerous diseases. If "such evidence is necessary to enable the claimed methods", as asserted on page 8 of the Office Action, then Applicants respectfully request identification of the statutory or case law that requires that they show success in "any and all" hosts. Applicants respectfully submit that they have made the required showing, and that this showing would be accepted by those skilled in the art for the methods now claimed.

For all of the above reasons, Applicants respectfully submit that the claims are enabled and that the specification as filed teaches a method which is effective in carrying out the claimed methods.

Rejections Under 35 U.S.C. § 102

Claims 1, 15 and 29 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by either Tang et al. (Nature, 1992) or Barry et al. (Biotechniques, 1994). This rejection is respectfully traversed.

The present invention, as recited in Claims 1, 15 and 29, is generally directed to methods for treating a host capable of generating an immune response by introducing to an antigen presenting cell within the host a particulate polynucleotide on which has been distributed a DNA fragment which expresses an antigenic protein or antigenic protein fragment; the result of this introduction to the antigen presenting cell is that the antigenic protein or fragment thereof is presented to the

membrane surface of the antigen presenting cell through the MHC class I pathway. An anti-tumor or anti-viral immune response is elicited as a result of this presentation, and tumor or virally-infected cells are destroyed. As will be understood by those skilled in the art, the presentation via the MHC class I pathway refers to the elicitation of a CTL response. In contrast, both Tang et al. and Barry et al. teach only antibody responses. The mechanism by which an antibody response is triggered is different from that in which a CTL response is elicited. Moreover, antibody responses are generally not effective against tumor cells or virally infected cells. Thus, the methods of Tang et al. and Barry et al. teach a different result.

In addition, both Tang et al. and Barry et al. teach the use of a gene gun, or biolistic device. Claim 29, however, is specifically directed to use of direct injection, and not a biolistic device. Thus, neither of the references teach the method of Claim 29.

To establish anticipation under 35 U.S.C. § 102, every element of a claim must be present in a single reference. (See, for example, *Jamesbury Corporation v. Litton Industrial Products, Inc.*, 225 USPQ 253 (Fed. Cir. 1985), a copy of which is enclosed.) Not every element in Claims 1, 15 and 29 is taught by the two references. Specifically, the references do not teach transfection of professional APCs or presentation of an antigenic protein or fragment thereof through the MHC class I restricted pathway by an APC. Delivery of genes to professional APCs and transfection of APCs is a unique facet of the present invention that is unreported in the art. Furthermore, neither of the references teach the inoculation of a mammalian host with a particulate polynucleotide by direct injection, as recited by Claim 29. Because the references do not teach these elements, Applicants respectfully submit that the present invention is not anticipated by the reference.

Claims 1, 15 and 29 were rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Hui et al. (Journal of Immunological Methods). This rejection is respectfully traversed.

First of all, it is noted that it is unclear whether the Hui reference is appropriately cited under 35 U.S.C. § 102(b). Although the paper bears a publication date of 1994, nothing indicates whether the publication occurred before

September 28, 1994, which is one year before the effective filing date of the current application.

In any event, Hui does not appear to teach every element of the claims. For example, Hui does not appear to teach delivery of particulate polynucleotides to antigen presenting cells. Hui discusses immunization through thigh muscle and spleen cells. Neither the immunization through thigh muscle, nor spleen cells, however, appeared to stimulate a CTL response. (See, Hui, page 151, column 2, "The thigh muscles were immunized. . . . After 20 days, no primary anti-H-2K^b CTL activity could be detected in the spleen cells."; and page 152, column 1, "As in the case of genetic immunization via the thigh muscles, the primary anti-H-2K^b activity detected was marginal. . . .") It was only after restimulation *in vitro*, that anti-H-2K^b activity was seen. In contrast, Applicants' methods of transducing antigen presenting cells results in elicitation of a CTL response *in vivo*. Along this line, Hui doesn't appear to show that their method has any anti-tumor or anti-viral immunity as claimed in the present methods. Hui reports that only an allo-antigenic response is elicited, and uses an artificial transplantation rejection antigen of questionable relevance to tumor or viral immunity. In addition, Hui does not show *in vivo* tumor or viral injection, APC transfection, or direct injection of particulate DNA. Finally, Hui is limited to use of a biolistic device, and therefore does not read on the methodology recited in Claim 29 for reasons stated above. Thus, several aspects of the presently claimed invention are not taught by Hui; because these aspects are lacking in the reference, it is not appropriately cited under 35 U.S.C. § 102(b).

Rejections Under 35 U.S.C. § 103

Claims 1, 15, 29, 44 and 59 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Weiner et al. (U.S. Patent No. 5,593,972) in view of either Tang et al. or Barry et al. This rejection is respectfully traversed.

Weiner appears to be directed to methods for using naked DNA to elicit an immune response. It is conceded in the Office Action that Weiner does not teach the use of DNA vaccines or particular polynucleotides for use in particle bombardment. A speculative sentence in Weiner, that their methods might have increased efficiency by use of particle bombardment, is cited as allegedly

overcoming the shortcoming of the reference. Applicants respectfully submit that a one-sentence speculation as to a manner in which efficiency may be increased does not render obvious the present invention. Moreover, the combination of Weiner with Tang and Barry, who are cited as teaching particle bombardment, does not overcome the shortcoming of the primary reference. More specifically, as noted above, Tang and Barry utilize particle bombardment in the elicitation of an antibody response, not a CTL response. Thus, combining the teachings of Tang and Barry with those of Weiner would lead one skilled in the art to conclude that use of particle bombardment would result in an antibody response. As noted above, Applicants' methods are clearly directed to the elicitation of a CTL response, which is decidedly different than an antibody response. In addition, Weiner does not appear to report transfection of antigen presenting cells. This is clearly claimed, however, by Applicants.

For a combination of references to be properly applied, the combination must suggest an improvement along the lines of the invention to those skilled in the art. (See, for example, *In re Sernaker*, 217 USPQ 1 (Fed. Cir. 1983), a copy of which is enclosed). Here, the Applicants cannot discern any suggestion whatsoever that the combination relied on in the Office Action would lead to the present method for eliciting an anti-tumor or anti-viral CTL response by use of particulate polynucleotides coated with DNA expressing an antigenic protein or fragment thereof. There is no teaching in Weiner of use of particulate DNA, or the introduction of particulate DNA to antigen presenting cells. There is no teaching in either Tang or Barry of the use of particle bombardment to elicit a CTL response. Therefore, the combination of the two references cannot be said to teach Applicants' claimed method of eliciting a CTL response, such that an anti-tumor or anti-viral immune response is elicited in a treated host. Furthermore, none of the references appear to teach use of direct inoculation, rather than use of a gene gun or biolistic device; direct injection is specifically recited in Claims 29, 44 and 59.

For all of the above reasons, Applicants respectfully submit that the references cited in the Office Action do not combine to render the present invention obvious.

New Claims

Applicants have added four new claims which further describe the present methods. More specifically, Claims 68 through 70 are directed to a method for transfecting antigen presenting cells. It is clear from the specification as filed that the present invention is directed to such a method. (See, for example, Example Sections 6, 7 and 8.) The examples demonstrate that the present methods result in antigen presenting cells that are transfected with DNA expressing an antigenic protein or fragment thereof. Such methods would have clear utility in the art of immunotherapy, both protective and therapeutic, for the reasons given throughout the specification. The two Falo Declarations submitted during prosecution further demonstrate that the current methods transfect APCs, as evidenced by the expression of GFP and/or LacZ by cells treated according to the present methods. In addition, none of the references cited in the Office Action appear to teach transfection or transduction of antigen presenting cells. Thus, it is submitted that the specification as filed supports Claims 68 through 70, and that these claims are free of the art.

Similarly, it is clear from the examples that Applicants have presented a method for inducing a CTL immune response in a host by transfection of APCs, as recited in new Claim 71. This transfection results in presentation of an antigenic protein or fragment thereof on the surface of the antigen presenting cell, which is in contact with the MHC class I pathway thereby eliciting a CTL immune response leading to the destruction of tumor cells. As discussed above, none of the references appear to teach methods for eliciting a CTL response that results in anti-tumor activity. In addition, the examples clearly show that such a response is elicited by the present methods. The Office Action concedes that anti-tumor activity is demonstrated in the current examples. (See Office Action, page 4.) Thus, Applicants respectfully submit that Claims 71 is enabled by the specification, and is free from the art.

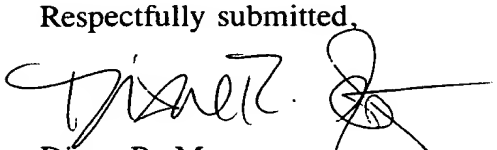
SUMMARY

For all of the above reasons, Applicants submit that the currently pending claims, including the newly added claims, are enabled by the specification, as filed; Applicants have presented evidence using art accepted animal models, and

that those skilled in the art can reproduce Applicants' methods to achieve the claimed results. In addition, it is submitted that the art of record does not anticipate or teach the claimed methods. Applicants respectfully submit, therefore, that the

above claims are in condition for a Notice of Allowance; such action is respectfully requested at an early date.

Respectfully submitted,



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